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PRIORITY DOCUMENT

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In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

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Signed Andrew Gersey

Dated 17 MAR 1998

Patents Form 1/77

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P

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference BIO DELIVERY 1

2. Patent application number *(The Patent Office will fill in this part)* **9703002.7** 13 FEB 1997

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if you know it)* 07155120001

If the applicant is a corporate body, give the country/state of its incorporation [SEE CONTINUATION SHEET].

4. Title of the invention
BIOREDUCTIVELY ACTIVATED DRUG TARGETING

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

FRANK B. DEHN & CO.
179 Queen Victoria Street
London
EC4V 4EL.

166001

Patents ADP number *(if you know it)*

FS1/77 JG 12/6/97

11 FEB 1997

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	1
Description	3
Claim(s)	1
Abstract	
Drawing(s)	5

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. DECLAN NAUGHTON

I/We request the grant of a patent on the basis of this application.

Signature Declan Naughton Date 10-02-'97

12. Name and daytime telephone number of person to contact in the United Kingdom DECLAN NAUGHTON 0171 377 7000 EXT 2947.

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Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

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d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

3. Full name, address and postcode of applicants

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BIOREDUCTIVELY ACTIVATED DRUG TARGETING

Field of the Invention

This invention belongs to the fields of pharmaceutical chemistry and the pathologies associated with hypoxia, and concerns bioreductive-drug conjugates which are intended for delivery of therapeutic compounds to hypoxic tissue. The compounds comprise a bioreductive group and a therapeutic agent.

Background of the Invention

Tissue hypoxia is well known to be associated with cancer and has been utilised as the rationale for a class of drugs known as bioreductives. In 1972 it was proposed that the low oxygen tension in solid tumours may be conducive to the reductive metabolism of a drug generating a compound more toxic than its parent. Reductive metabolism means the addition of electrons to the compound with the addition of electrons effectively activating the compound. The drug acts as a substrate for cellular reduction enzymes. Most 'bioreductive drugs' that have been developed subsequently exhibit an optimum 'trapping' potential when hypoxia is profound (< 12mm Hg) which is the basis of their selectivity for cancerous (hypoxic) as opposed to normal (normoxic) tissues.

Bioreductives can be envisaged to belong to three classes:-

i) Quinones; some indoloquinones are good substrates for DT diaphorase, a fairly ubiquitous enzyme, and their activation is indifferent to tissue oxygen tension. Others having differential cytotoxicity under hypoxic conditions can also be substrates for other reductases.

ii) Nitro-heterocycles; these compounds bind irreversibly within hypoxic cells following bioreduction both *in vitro* and *in vivo*. Various intracellular sites may be involved but DNA is a known binding site for reduced nitroheterocycles. The level of binding is related to both pO_2 and the reductase level. P450 reductase appears to be particularly important in the activation of 2-nitroimidazoles. Bioreduction occurs via the nitro-group and it is the resultant metabolite which is believed to bind to intracellular DNA.

iii) N-oxides; Under anaerobic conditions tirapazamine is reductively activated by cytochrome P450 and cytochrome P450 reductase. The reduced products cause DNA strand breaks. The aliphatic N-oxide, AQ4N, reduced solely by cytochrome P450, in the absence of oxygen is reduced to AQ4, an agent with high affinity for DNA. This process involves a distortion of the DNA double helix and is associated with chromatin aggregation and compaction. These distortions result in inhibition of DNA and RNA polymerases and topoisomerases Type I and II.

Bioreductives have selective cytotoxic effects in tissues with enhanced reductase activity. This may be a consequence of hypoxic metabolism, reduced oxygenation or both.

We propose to link a bioreductive compound to another active drug. The originality lies in taking

advantage of the high level of reductases in an hypoxic environment as a targeting device, but not formulating an active cytotoxic agent. An ideal compound would have the capacity to pene poorly perfused tissue, would only release the active drug in an hypoxic environment and v possess no cytotoxic activity. In a clinical setting tissues become ischaemic (hypoxic/poorly perfused) if the blood supply is reduced as in atherosclerosis/ diabetes/ following transplantation or if an inflammatory or cancerous response leads to the tissue to either physically or metabolically outgrow its vascular supply. Many known drugs could have enhanced therapeutic effects if selectively delivered to ischaemic tissue. For example, i) after a cerebrovascular attack, cerebral perfusion is reduced and the brain suffers an inflammatory response. The linkage of a vasodilator (such as a nitric oxide generator) or an anti-inflammatory agent (such as a steroid) to a bioreductive should enhance the 'therapeutic index' of the active agent, a non-steroidal anti-inflammatory agent, a steroid or a nitric oxide inhibitor, ii) in chronic synovial inflammation (rheumatoid arthritis) synovial tissues may be profoundly hypoxic (< 12 mm Hg) and perfusion is poor. The ability to link an anti-inflammatory agent such as a non-steroidal anti-inflammatory agent, steroid or a nitric oxide inhibitor would again enhance the therapeutic index of the active agent, iii) in transplantation and rejection both ischaemia and the immunological-inflammatory response contribute to and compound tissue hypoxia. A bioreductive linked to a vasodilator/anti-inflammatory or immunological suppressant would have therapeutic activity again by enhancing the therapeutic index of the active agent.

Prior art relating to bioreductive compounds

A number of potential hypoxia-based drug targeting systems have been described.

Mitomycins

Mitomycins are indoloquinones which contain an aziridine ring. Reductive activation of the quinone generates the electron rich hydroquinone resulting in bond rearrangement and ejection of the methoxy group. This process generates a C_1 methide capable of alkylating guanine amino groups in DNA and regenerating the hydroquinone. Further bond rearrangement leads to ejection of the carbamate, thus generating a second alkylating moiety for DNA bonding (Scheme 1).

Quinone methides

Alkenyl-substituted quinones which are functionalised with a leaving group at the 3 position on the side chain can be reduced *in vivo* to the hydroquinone. This reduction converts the quinone (an electron sink) into an electron-rich hydroquinone. Subsequent intramolecular bond rearrangement results in the elimination of the leaving group, a process which generates the cytotoxic alkylating agent (Scheme 2).

Nitroaryls

Nitrobenzyl species bearing a number of leaving groups are known to undergo fragmentation following one electron reduction. These include nitrobenzyl quaternary mustards for delivery of the

mustard cytotoxin to hypoxic tumours (Scheme 3).

Quinone lactonisation

A delivery system based on quinone propionic acid has been described (Scheme 4). In this delivery system the benzoquinone acts as the trigger and the propionic acid moiety acts as the linker. The propionic acid linker group allows for conjugation with both amine moieties (e.g. enzyme inhibitors) and alcohols (e.g. steroids). Thus a wide range of drugs may be delivered using this system.

Two electron reductive activation of the benzoquinone trigger facilitates intramolecular cyclisation generating the stable lactone, a process which results in elimination of the drug to the site of hypoxia (Scheme 4). This process is inhibited by oxygen.

However, this compound is a potential alkylating agent and would not be suitable for use as a non-toxic delivery system. Furthermore, in aqueous solution in the absence of a reducing agent these compounds are very unstable and undergo degradation. The instability of this prodrug system in aqueous solution precludes its use for drug delivery.

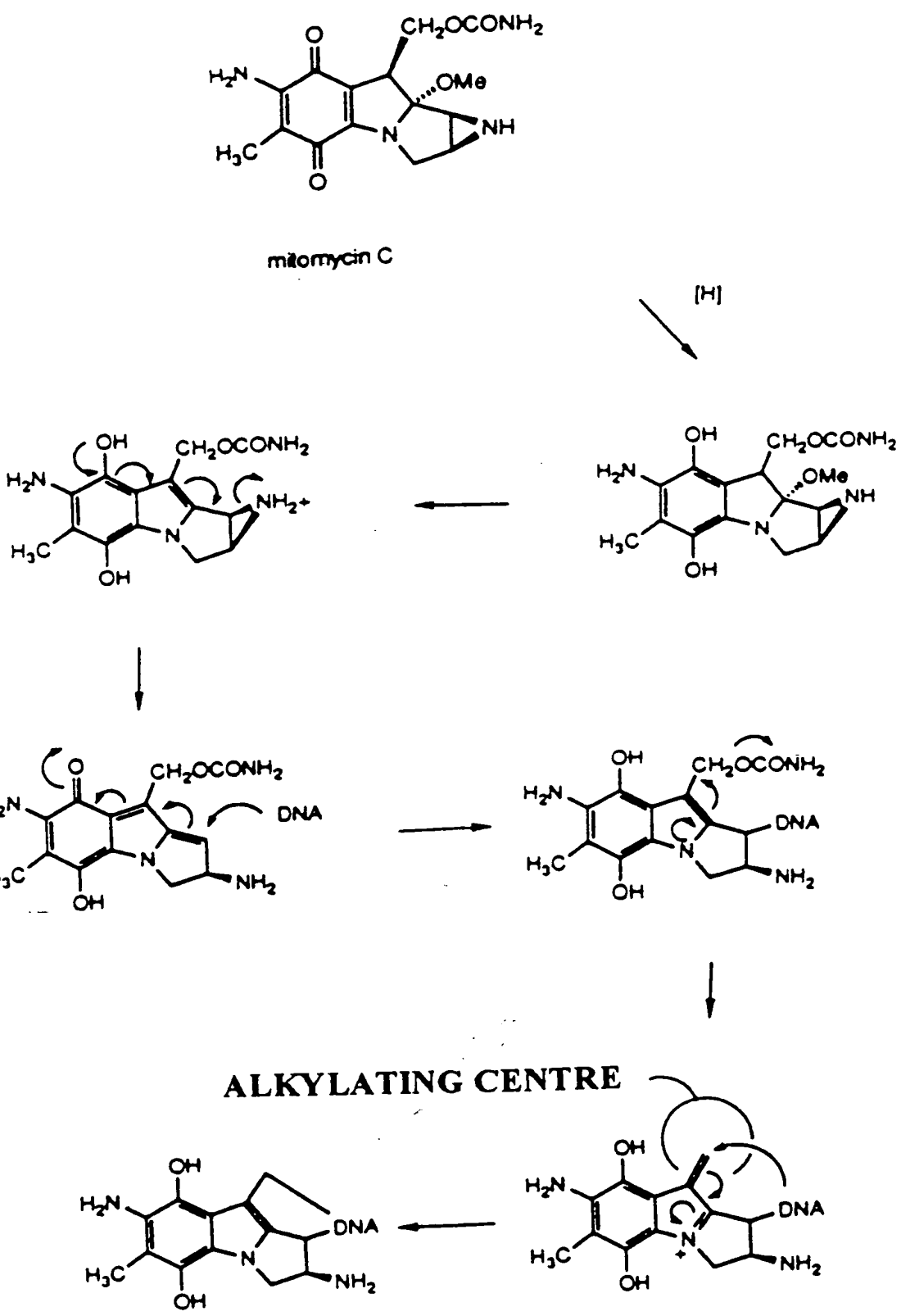
In order to circumvent the cytotoxicity associated with the carriers described in delivery systems it is possible to include a nucleophile in the carrier molecule. Thus, the proximity of the nucleophile ensures that intramolecular alkylation occurs in preference to alkylation of biomolecules such as DNA (Scheme 5).

Claim

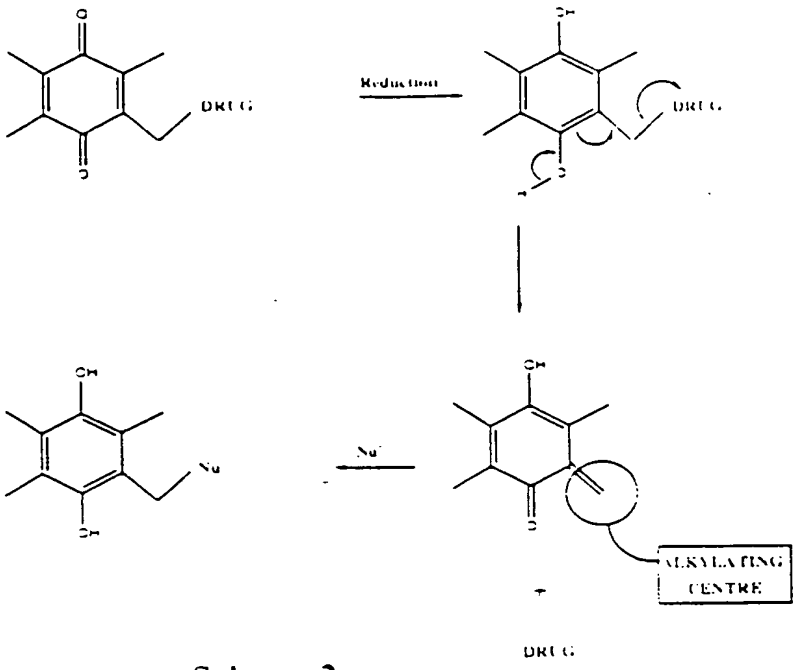
A compound comprised of two components A and B wherein A and B are stably conju in an oxygenated environment and are characterised in that A is not cytotoxic and B when conjugated to A is not cytotoxic and does not become cytotoxic. On reductive activation of A, A and B detach and A reacts with itself to become stable (Figure 1).

Example

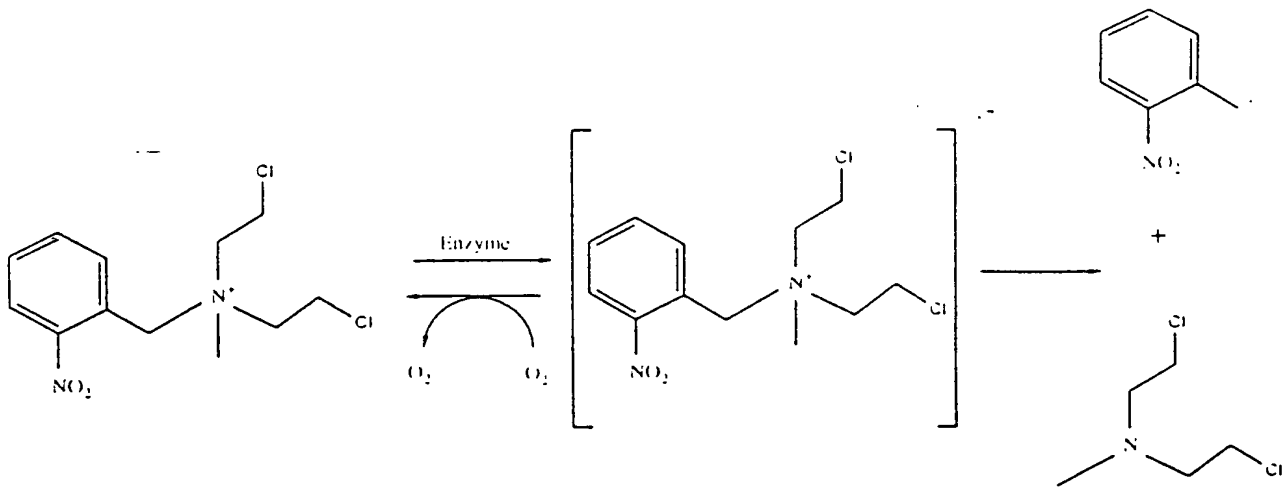
A quinone may be constructed so as to contain a nucleophilic moiety which will favour an intramolecular alkylation. Suitable electron rich groups include oxygen and nitrogen atoms. Mitomycin C alkylates DNA via nucleophilic attack of the guanine amine moiety. Thus, inclusion of an amine group within the bioreductive carrier molecule will favour intramolecular alkylation rendering a non-cytotoxic product upon release of the drug at the site of hypoxia. Scheme 5 illustrates such a molecule. Inclusion of the amine adjacent to the alkylating moiety ensures that site-specific intramolecular alkylation occurs.



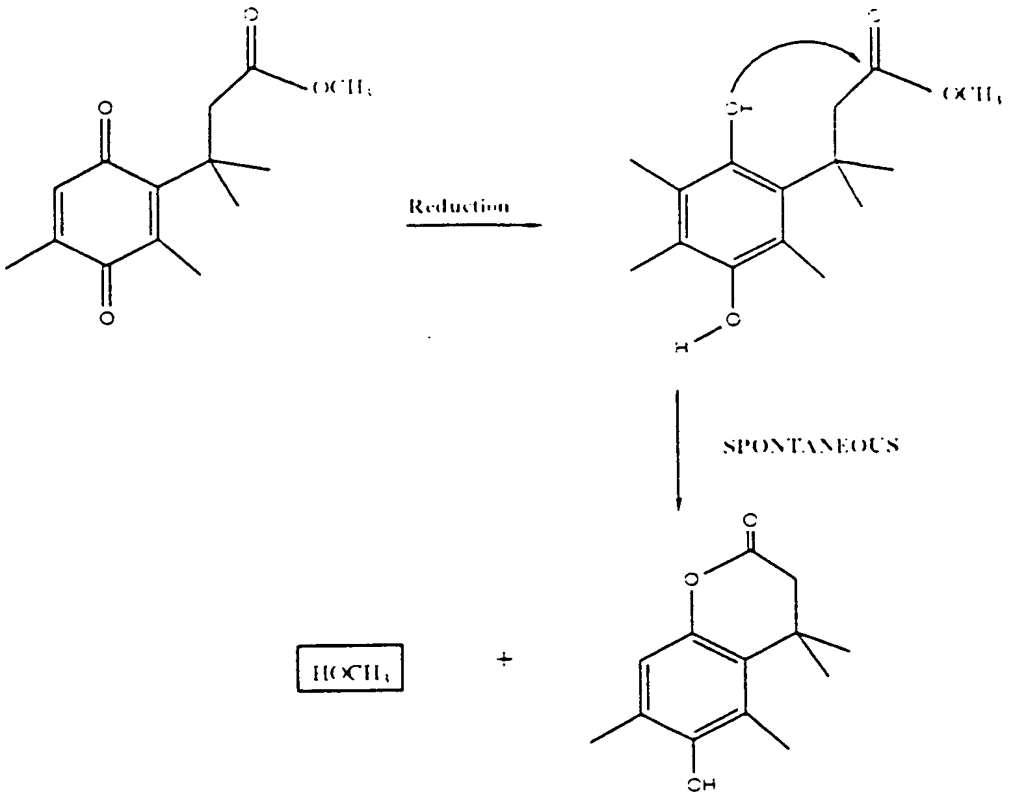
Scheme 1



Scheme 2



Scheme 3



Scheme 4

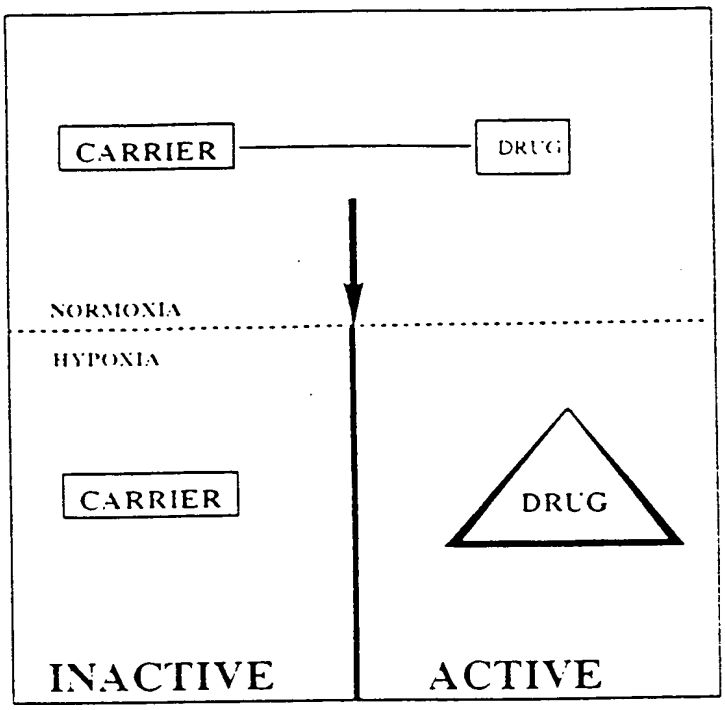
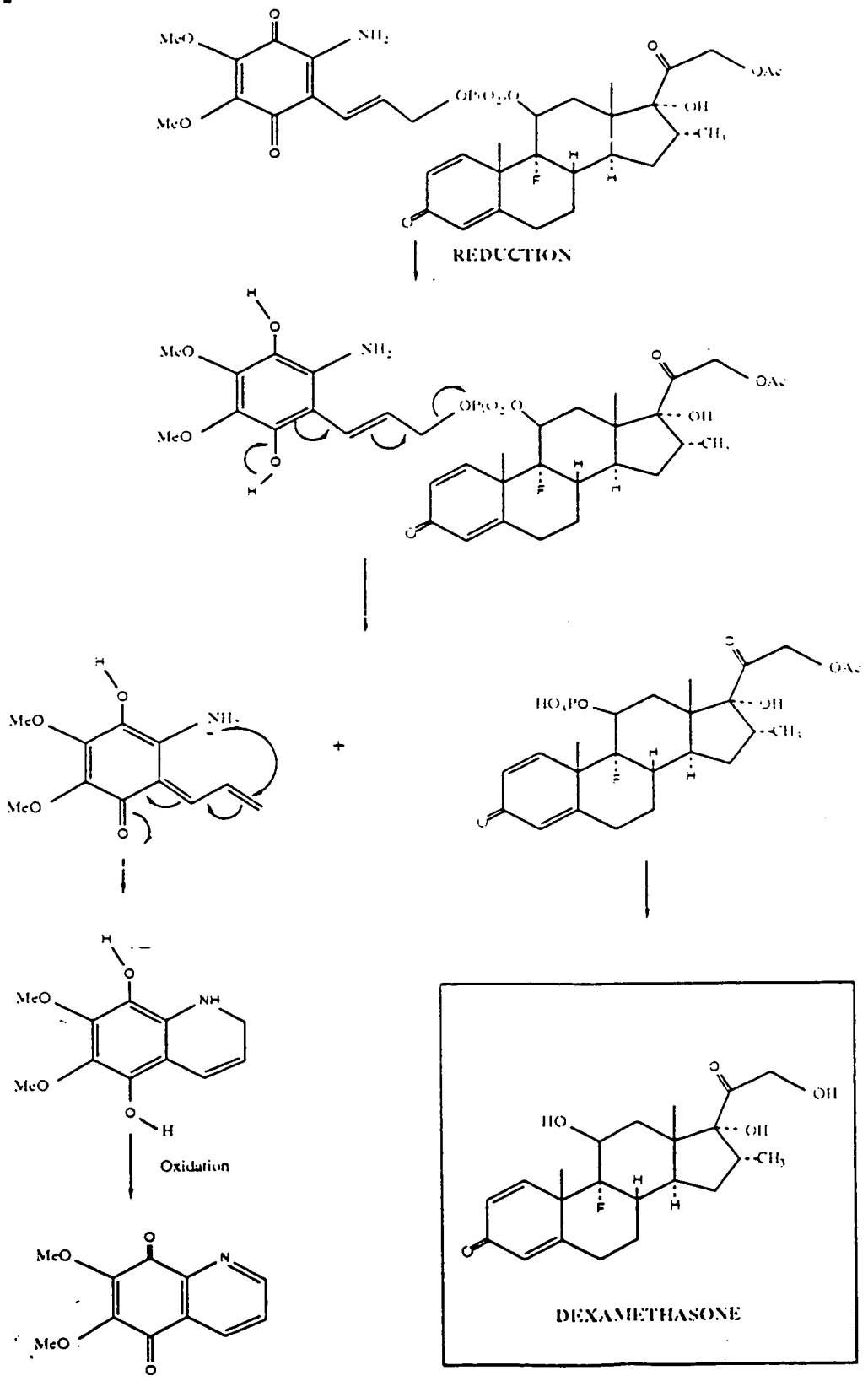

CARRIER
DRUG
NORMOXIA
HYPOXIA
CARRIER
DRUG
INACTIVE
ACTIVE

Figure 1

BIOREDUCTIVE-GLUCOCORTICOID: ACTIVATION



Scheme 5

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Frank B. Dehn.